Optimization of Naphthyridones into Selective TATA-Binding Protein Associated Factor 1 (TAF1) C-Terminal Bromodomain Inhibitors

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ABSTRACT: Bromodomain containing proteins and the acetyl-lysine binding bromodomains contained therein are increasingly attractive targets for the development of novel epigenetic therapeutics. To help validate this target class and unravel the complex associated biology, there has been a concerted effort to develop selective small molecule bromodomain inhibitors. Herein we describe the structure-based efforts and multiple challenges encountered in optimizing a naphthyridone template into selective TAF1(2) bromodomain inhibitors which show promise for the future development of small molecules to interrogate TAF1(2) biology. Key to this work was the introduction and modulation of the basicity of a pendant amine which had a substantial impact on not only bromodomain selectivity, but also cellular target engagement.

The 61 human bromodomains are comprised of 48 typical bromodomains where two conserved amino acid residues, asparagine and tyrosine, form a direct and a water-mediated hydrogen-bond interaction to an acetyl-lysine (KAc) residue.[1] This interaction drives the recruitment of cellular transcriptional machinery to a specific epigenetic histone mark and regulates gene expression.[2] These epigenetic reader modules have emerged as an new exciting class of therapeutic targets for a variety of diseases including immune disorders, metabolic disease and oncology.[3,4] The majority of research to date has been focused on the dual bromodomain containing bromodomain and extra terminal (BET) family of proteins and a number of molecules targeting the BET proteins are currently undergoing oncology clinical trials.[5] Driven by the profound and fundamental biology associated with the BET proteins, interest in the 53 non-BET bromodomain containing proteins (BCPs) has increased. To help understand the role these bromodomains play in modulating healthy and disease states, a number of small molecule inhibitors and chemical probes have been developed and disclosed.[6] Used in conjunction with other techniques such as gene knockdown or knockout, small molecule chemical probes can provide insight into the role of individual protein domains present in multidomain proteins such as BCPs.[7,8] To give increased confidence in phenotype assignment, access to and use of multiple well characterized inhibitors of different chemotypes is beneficial.[9] Although there are a significant number of reported small molecule inhibitors and probes for bromodomains, several lack the sufficient breadth of chemical equity for robust target validation.

TAF1 and the highly related TAF1L are multidomain proteins each of which contains two kinase domains, a histone acetyl transferase domain and two bromodomains termed TAF1(1) / TAF1L(1) (N-terminal bromodomain) and TAF1(2) / TAF1L(2) (C-terminal bromodomain). TAF1 and TAF1L are associated factors of the TATA-binding protein (TBP), a subunit of transcription factor IID (TFIID), which plays a critical role in RNA polymerase transcription. TFIID acts as a scaffold for the assembly of the pre-initiation complex formed prior to transcription, coordinates the alignment of RNA polymerase with DNA and helps facilitate binding to DNA.[10,11] Dysregulation of TAF1 has been associated with a number of diseases across oncology and neurology: Overexpression of TAF1 has been shown to increase androgen receptor activity several fold resulting in the progression of prostate cancer and it has been proposed that TAF1 mutation may play a role in colorectal and gastric cancers.[12,13] TAF1 bromodomain mutations have also been shown to contribute to the phenotypes displayed across multiple neurodegenerative X-linked syndromes, including intellectual disability and facial dysmorphology.[14]

While the involvement of TAF1 within such disease has been established, the role of the TAF1 bromodomains is less clear. To this end, in 2014 Bradner and co-workers reported the first sub-micromolar TAF1/TAF1L bromodomain small molecule inhibitor, UMB-32 (**1**) with comparable activity against BRD4 (Figure 1).[15]

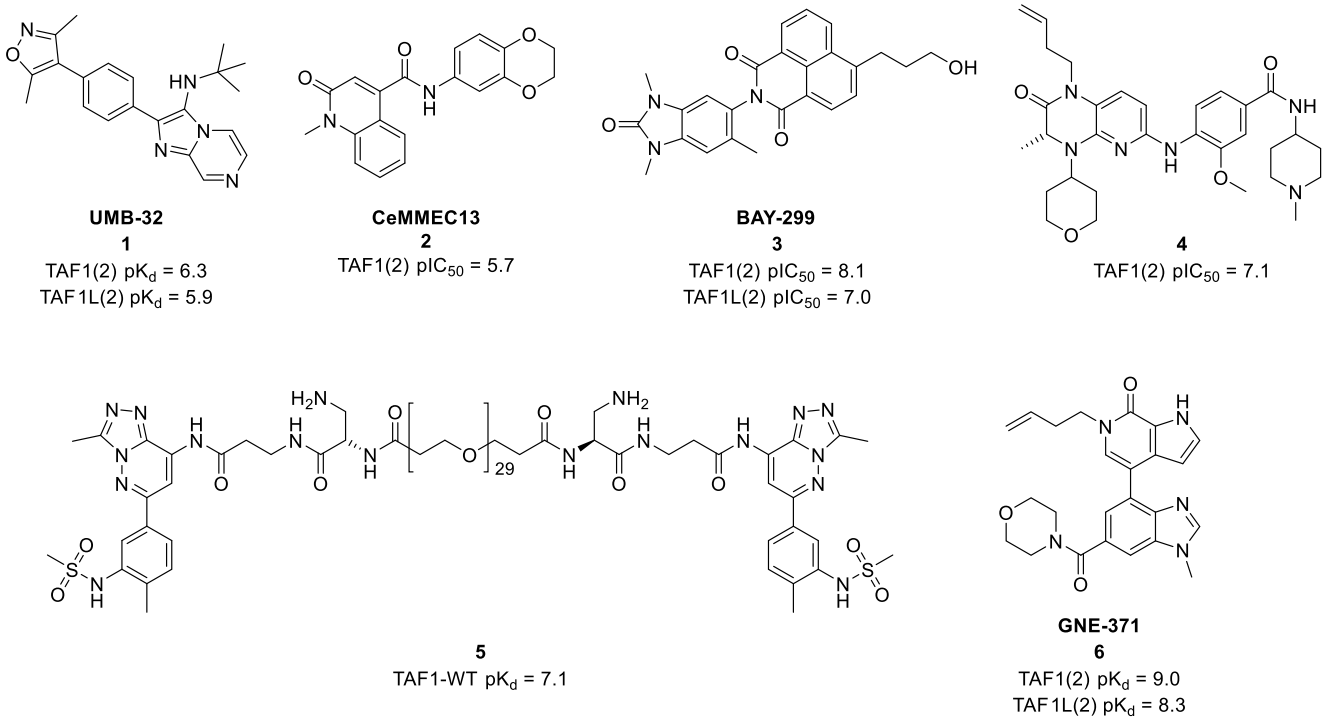


Figure 1. Disclosed TAF1/TAF1L inhibitors and their reported bromodomain affinities

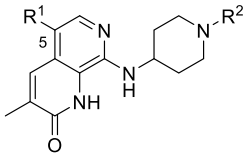
Since this demonstration of the small molecule tractability of TAF1(2)/TAF1L(2), there have been several reports in the patent and peer-reviewed literature of inhibitors. CeMMEC13 (**2**) was disclosed by Sdelci and co-workers as a TAF1 inhibitor,[16] and researchers from Bayer developed BAY-299 (**3**), a potent inhibitor of both TAF1(2)/TAF1L(2) and BRPF2.[17] Compound **4** was developed from polo-like kinase 1 inhibitor BI-2536 and demonstrates >120 fold selectivity for TAF1(2) over BRD4(1).[18] Bifunctional molecule **5** was designed to bind to both bromodomains of TAF1 bivalently and demonstrated significant affinity gain over monovalent ligand binding.[19] Additionally, a bivalent molecule designed to inhibit the BET bromodomains was also reported to inhibit TAF1(2), although it is unclear whether the TAF1 inhibition is bivalent or not.[20] Selective and potent TAF1(2)/TAF1L(2) chemical probe GNE-371 (**6**) was recently developed by Genentech and Constellation Pharmaceuticals.[21] The 1-butenyl group in GNE-371 functions as the KAc methyl mimetic and takes advantage of the differential stability of conserved bromodomain water networks to induce selectivity at TAF1(2) through rearrangement and stabilization of the waters in the KAc binding site.[22] To aid TAF1 target validation efforts, we sought to develop a structurally diverse, potent and selective small molecule inhibitor of the second bromodomain of these targets. Selectivity is an important consideration when linking a biological phenotype to small molecule inhibition.[7] Due to the profound biological phenotype associated with BET family bromodomain inhibition,[2] a minimum target of >100 fold selectivity was set, together with >30 fold over other non-BET bromodomains. Herein, we report the optimization of a naphthyridone template into potent and cell active inhibitors of TAF1(2).

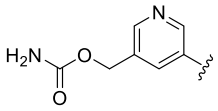
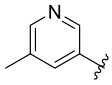
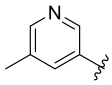
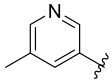
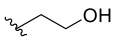
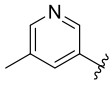
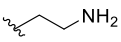
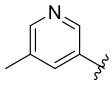
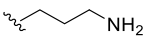
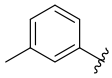
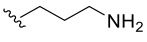
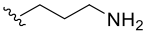
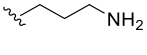
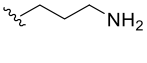
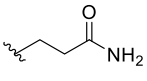
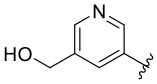
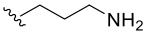
TAF1 and TAF1L both contain tandem bromodomains with differing levels of homology between the four domains (Figure 2a). The N-terminal bromodomains in both proteins only differ by a single amino acid and the C-terminal bromodomains are 97% identical. In contrast, the C and N terminal bromodomains within TAF1 and TAF1L show significant differences to each other (37-39% identity). In particular, a change in gatekeeper residues from leucine in TAF1(1)/TAF1L(1) to tyrosine in TAF1(2)/TAF1L(2) creates a fundamentally different binding pocket and impacts access to the lipophilic WPF shelf.[23] Interestingly, there are no commercial assays for TAF1(1) or TAF1L(1), so it is unclear if the molecules described in the course of this work are selective over the first bromodomain or not and the majority of published reports also focus on the C-terminal bromodomains of these proteins. Due to the high homology between TAF1(2) and TAF1L(2) it was not expected that selectivity between the bromodomains would be achievable and as such, was not sought or monitored.

To identify TAF1(2) inhibitors, a screen of molecules from the GSK compound collection known to or predicted to contain acetyl lysine mimetics was carried out against TAF1(2) using a Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) competition assay. A TR-FRET assay against the N-terminal bromodomain of BRD4, termed BRD4(1) was also run as an anti-target as a representative member of the BET family. While several TAF1(2) active compounds were identified, the majority were also at least as potent against BRD4(1). Naphthyridone **7** stood out amongst the hit compounds with 8 fold bias over BRD4(1) and an encouraging TAF1(2) ligand efficiency (LE) of 0.31 (

Table 1). Compound **7** had been accessed during a previous research effort that resulted in a high quality ATAD2 chemical probe and the template was also used to develop a tool for the first bromodomain of the BET bromodomains.[24-27] While activity against TAF1(2) with this template had been identified previously,[25,27] compound **7** demonstrated an intriguing 10 fold selectivity over ATAD2. Compound **7** was highly polar (ChromLog$D_{pH7.4}$: 0.3) which translated into good aqueous solubility, excellent LLE (6.5) and poor (<3 nm/s) passive permeability as measured by an artificial membrane permeability assay (AMP).

Table 1. Profiling of Compounds 7-18[a]



Compound	R^1	R^2	TAF1(2) pIC_{50}	TAF1 (2) LE	BRD4(1) pIC_{50} (selectivity)	ATAD2 pIC_{50} (selectivity)	Chro mLog $D_{pH7.4}$	CLND (µg/mL)	AMP (nm/s)
7		H	6.8	0.31	5.9 (×8)	5.8 (×10)	0.3	≥144	<3
8		H	6.7	0.35	5.6 (×13)	5.7 (×10)	1.2	≥72	<3
9		Me	6.5	0.33	5.7 (×6)	5.6 (×8)	1.5	117	<3
10			6.3	0.30	5.6 (×5)	5.9 (×3)	1.1	≥176	<3
11			7.1	0.33	5.2 (×80)	5.7 (×25)	1.6	68	<3
12			7.3	0.33	5.6 (×50)	6.0 (×20)	1.2	29	11
13			6.0	0.27	5.7 (×2)	5.0 (×10)	2.8	22	13
14			6.2	0.29	4.9 (×20)	5.0 (×16)	3.1	49	<10
15			6.4	0.30	4.8 (×40)	4.9 (×32)	0.8	75	<10
16			7.5	0.35	5.2 (×200)	4.7 (×630)	1.2	71	<5
17			<4	-	5.2 (-)	n.d.	1.4	≥206	<7
18			7.5	0.33	5.6 (×80)	6.2 (×20)	0.2	34	8

[a]LE = (1.37 × pIC_{50})/heavy atom count. n.d. = not determined. For statistics, see Supporting Information, Table S1.

Removing the primary carboxamide to reduce the polarity was tolerated at TAF1(2), together with a concomitant increase in LE, although no measurable increase in passive permeability was observed (compound **8**). Capping the piperi-

dine NH with a methyl group with compound **9** to reduce the number of hydrogen bond donor also had little effect on the bromodomain activity profile or the low passive permeability. Modelling of compound **9** into apo TAF1(2) (PDB: 3UV4) indicated that the piperidine ring may offer a vector for further interactions to boost potency, in particular with Asp1539 (TAF1(2) numbering, vide infra). A pendant alcohol was broadly tolerated with compound **10**, whereas primary amine **11** gave a far improved profile indicating for the first time that high levels of selectivity over BRD4(1) and ATAD2 while retaining TAF1(2) activity was possible from the naphthyridone template. The selectivity over ATAD2 also improved to 25-fold, though not to the same level as BRD4(1). BRD4(1) and ATAD2 all contain an acidic group in the same 3-dimensional space as Asp1539 in TAF1(2), so it is unclear as to why the presence of the amine in compound **11** is only beneficial for TAF1(2) potency. Homologation of the primary amine to compound **12** gave a similar profile to compound **11**, although a minor improvement in passive permeability was observed.

(a)

	TAF1(1)	TAF1L(1)	TAF1(2)	TAF1L(2)
TAF1(1)	100	98	39	37
TAF1L(1)	98	100	39	37
TAF1(2)	39	39	100	97
TAF1L(2)	37	37	97	100

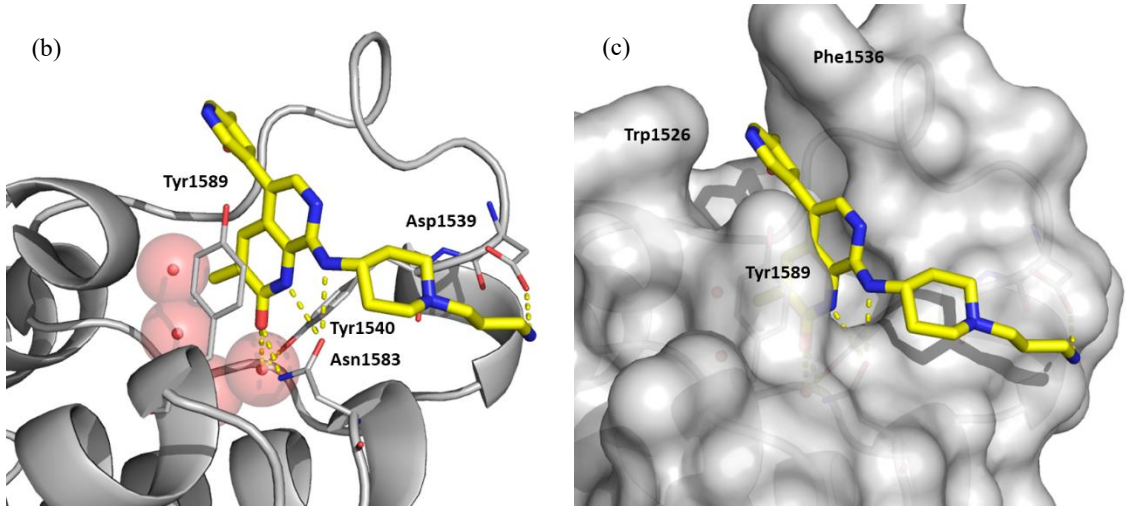


Figure 2. (a) Table comparing % sequence identify between TAF1(1, 2) and TAF1L(1, 2); (b) X-ray crystal structure of **18** (yellow) bound to human TAF1(2) (grey) (PDB: XXXX); (c) X-ray crystal structure of **18** (yellow) bound to human TAF1(2) (grey) with a protein surface (grey) (PDB: XXXX).

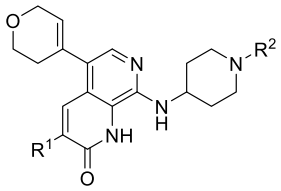
The 5-position pyridine ring was a preferred group on the naphthyridone template for ATAD2 activity due to its displacement of a water atom and subsequent hydrogen bonding interaction with Asp1014 (ATAD2 numbering) by the ring nitrogen.[24] In order to modulate the persistent ATAD2 activity of this template, replacement of this pyridine ring to remove this interaction was prioritised. Pyridyl replacement with a benzene ring with **13** was not well tolerated at TAF1(2) with a complete erosion of selectivity over BRD4(1) observed. Similarly, a cyclohexyl ring and a tetrahydropyran (compounds **14** and **15**) decreased the activity against TAF1(2), BRD4(1) and ATAD2 indicating that sp3 substitution is not well tolerated in the naphthyridone 5-position. However, restoring some sp2 hybridization with dihydropyran **16** gave a boost in TAF1(2) potency without a concomitant increase in BRD4(1) or ATAD2 potency resulting in a promising bromodomain selectivity profile. This finding confirmed the design hypotheses that the 5-position pyridine ring was at least partly responsible for persistent ATAD2 potency observed on the template to date. Highlighting the importance of the amine moiety, primary carboxamide **17** ablated activity at TAF1(2).

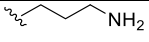
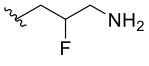
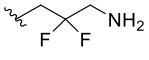
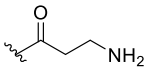
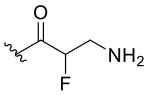
Initial attempts to obtain crystallography of naphthyridone compounds in complex with TAF1(2) were unsuccessful, however, a structure of related compound **18** bound to human TAF1(2) (pIC_{50}: 7.5) was ultimately achieved (**Error! Reference source not found.**b, c). As expected from the binding pose of analogues with ATAD2,[24] the carbonyl group functions as the KAc mimetic, forming the canonical direct hydrogen bond to Asn1583 and a water mediated interaction with Tyr1540. Additional hydrogen bond interactions between the

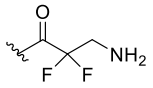
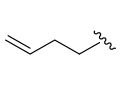
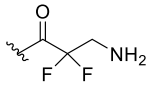
naphthyridone NH and the 8-position NH to Asn1583 completes the tridentate interaction to the conserved residue. The methyl group of the naphthyridone core acts as the KAc methyl mimetic, protruding towards the conserved water molecules in the binding site. Tyr1589, the gatekeeper residue, has rotated 18° compared to the published apo structure (PDB: 3UV4) to make a face-to-face interaction with the bicyclic core.[28] Confirming the design hypothesis, the flexible primary amine sits on the protein surface and makes a salt bridge to Asp1539 which has shifted 0.7 Å from the apo structure to make this interaction. The 5-position pyridine sits with a 60° dihedral angle against the WPF stack at the edge of the ZA channel and drives substantial reorganization of both Trp1526 and Phe1536 compared to the apo structure to accommodate. Whereas the pyridine nitrogen points into bulk solvent, the pendant alcohol makes a direct hydrogen bonding interaction with the backbone NH of Asn1533 and a through-water interactions to Pro1531, His1530 and Pro1527. It should be noted that these hydrogen bonding interactions between the pendant alcohol and the protein contributes little to TAF1(2) potency as seen by comparing the similar activity of matched molecular pair **12** and **18** (Table 1). The narrow nature of the channel that the 5-position substituent protrudes into helps explain the drop of potency upon substitution with an sp3 carbon: presumably the fully saturated ring system is not tolerated due to an increased dihedral angle which drives steric clashes with the protein (**Error! Reference source not found.**c).

Naphthyridone **16** showed an exciting bromodomain selectivity profile with encouraging solubility. However, the measured passive permeability was low (<5 nm/s), attributed to the dibasic nature of the molecule, indicating that bromodomain target engagement in a cellular system would be unlikely (predicted pKa's: 9.9 primary amine, 7.7 piperidine). To prioritise new design hypotheses, a GSK proprietary *in silico* predictive permeability model was evaluated against measured passive permeability data for 570 structurally related naphthyridones, but unfortunately, the model was not usefully predictive for this chemotype.

Table 2. Profiling of Compounds 16 and 19-24[a]



Compound	R^1	R^2	TAF1(2) pK_i	TAF1(2) LE	BRD4(1) pK_i (selectivity)	Chrom Log$D_{pH7.4}$	CLND (µg/mL)	AMP (nm/s)	Predicted pKa's
16	Me		9.4	0.44	5.7 (×5000)	1.2	71	<5	9.9, 7.7
19	Me		9.0	0.41	<5.3 (>×5000)	1.6	116	<10	8.6, 6.3
20	Me		7.3	0.32	<5.3 (>×100)	2.9	≥196[b]	220	7.3, 5.8
21	Me		8.9	0.41	6.1 (×630)	1.3	≥386	<3	9.1, 5.8
22	Me		9.7	0.43	6.2 (×3160)	1.7	≥201	<6.5	7.8, 5.8

23	Me		9.1	0.39	<5.3 (×6410)	2.7	24[b]	62	6.5, 5.7
24			6.7	0.26	<5 (>×50)	3.9	28[b]	176	6.5, 5.7

[a]LE = (1.37 × pKi)/heavy atom count. For statistics, see Supporting Information, Table S2; [b]CAD solubility

In contrast, a regression analysis of 30 calculated physicochemical descriptors of the naphthyridone compounds in the GSK database revealed a dependence of permeability on the pKa of the most basic unit, with passive permeability through an artificial membrane >100 nm/s more likely for molecules with pKa <9.5. To take advantage of this finding, a series of molecules were designed targeting reduction of the basicity of both basic centers, profiled and only those with a predicted pKa <9.5 taken forward into synthesis. A further consideration was conformational restriction of the flexible propylamine chain to improve potency and/or selectivity.

To speed up data generation across multiple bromodomains, a switch was made to utilize BROMOscan assays (pK_i values) as opposed to the internal TR-FRET assays (pIC_{50} values) that had been used to guide the chemistry to this point.[29] Due to the presence of the 5-position dihydropyran which had a substantial impact on ATAD2 potency, activity against this bromodomain was no longer routinely measured. It is of note that the BROMOscan potency was routinely higher than the corresponding TR-FRET data. This has been observed previously and may be due to sample preparation methods, alternative protein constructs, and/or different detection systems.[27]

Addition of fluorine to both restrict conformation and reduce the pKa of basic groups in a predictable manner is a commonly used method in medicinal chemistry.[30] To modulate the basicities of both amines simultaneously, substitution of the 2-carbon of the propylamine chain was targeted (Table 2). Monofluorination with racemic **19** was tolerated at TAF1(2), albeit without improved permeability. Double fluorination to give compound **20** did show the desired levels of passive permeability (220 nm/s), unfortunately at the expense of TAF1(2) potency. Carboxamide **21** showed high levels of TAF1(2) potency together with a drop of selectivity over BRD4(1). The low passive permeability is presumably related to the basic amine remaining. Adding one fluorine to give racemic α-fluoro **22** gave the most potent compound of the series at TAF1(2), but despite a pKa: 7.8, poor passive permeability was still observed. To complete the set of compounds, α-difluoro **23** was made and to our surprise nM activity at TAF1(2) was still achieved. Excellent selectivity over BRD4(1) was retained and measurable levels of permeability were observed in the passive permeability assay. Although the design hypotheses that reducing the predicted pKa would drive passive permeability was ultimately correct, the level by which the basicity needed to be reduced had been underestimated. The data indicated that for this set of compounds, meaningful passive permeability was only observed with a predicted pKa ≤7.3.

Restricted docking of the matched molecular pair **20** and **23** into the crystal structure of TAF1(2) from the complex with **18** was carried out to give insight into the difference in activity at TAF1(2) (Figure 3). As constrained by the docking protocol, both molecules maintained the tridentate hydrogen-bonding interaction with Asn1683 and the through water interaction with. Tyr1540 observed with the crystal structure of compound **18** bound to TAF1(2) (Figure 2b). The dihydropyran is accommodated in the cleft between Trp1526 and Phe1536 with a predicted 62° dihedral angle, like that observed with compound **18** bound to TAF1(2) (Figure 3b and c). The pendant amine for both molecules was able to maintain the interaction with Asp1539, however the presence, or not, of the amide has a drastic impact on the orientation of the fluoro groups (Figures 3c and d).

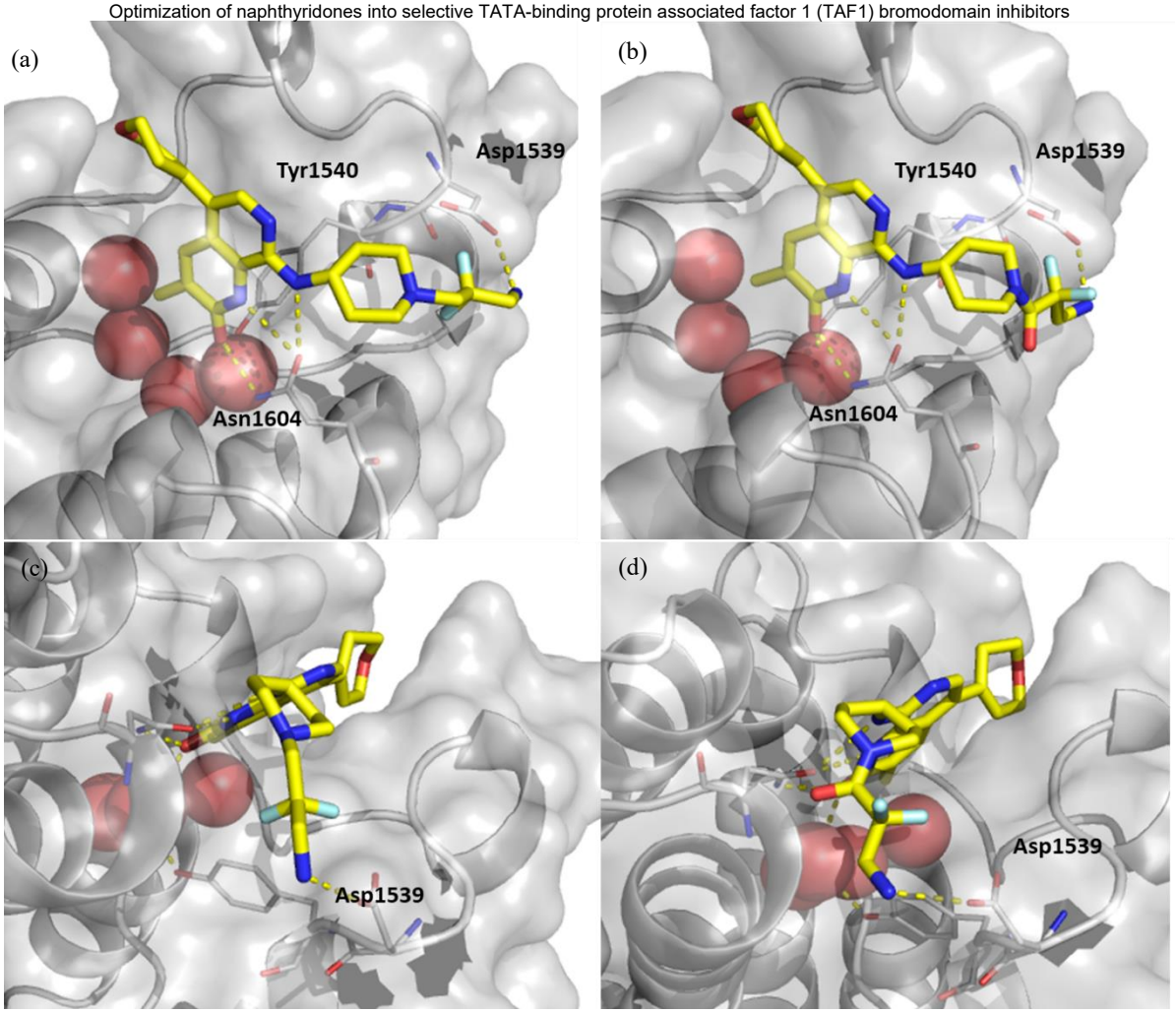


Figure 3. Docking of a) compound **20** (yellow) into TAF1(2) (grey); b) compound **23** (yellow) into TAF1(2) (grey); c) compound **20** (yellow) into TAF1(2) (grey) viewed from an alternative angle; d) compound **21** (yellow) into TAF1(2) (grey) view from an alternative angle using Glide (Schrodinger Inc) 2018.3. PDB: XXXX was used for all dockings.

When the amide is present, a conformation where one fluorine sits *anti* to the carbonyl group is adopted resulting in the fluorine groups protruding away from the protein surface.[31] Presumably this flip of the fluorine groups predicted for compound **23** relieves an unfavorable interaction with the protein surface that impacts the TAF1(2) activity of compound **20**.

The knowledge built around the template binding to TAF1(2) demonstrated that the naphthyridone methyl group functioned as the acetyl lysine methyl mimetic (Figure 2b). It has been previously demonstrated that TAF1(2) is able to recognize not just methyl acetyl lysine, but also atypical crotonyl and butyryl substituted acetyl lysines.[32] Genentech and Constellation Pharmaceuticals have elegantly utilized this facet of the TAF1(2) bromodomain with the 1-butenyl group as an atypical acetyl lysine methyl mimetic on a pyrrolopyridinone scaffold to induce TAF1(2) selectivity through the rearrangement and stabilization of the KAc binding site water molecules.[21,22] This approach was also successfully applied to a dihydropyridopyrazine scaffold in the development of dual BET, TAF1(2) inhibitor **4**.[18] Overlaying the published structure of GNE-371 bound to TAF1(2) with **18** bound to TAF1(2) indicated that the methyl groups occupied approximately the same space, even if the angle of the C-C bond was slightly different (Supporting information Figure S1). Accordingly, compound **24** bearing a 1-butenyl acetyl lysine methyl mimetic was accessed. Despite the successful translatability of the atypical acetyl lysine methyl mimetic approach for BRD7/9 bromodomain inhibitors across multiple chemotypes,[33] with the naphthyridone template, an unexpected >200 fold drop in TAF1(2) potency was observed making it challenging to understand if selectivity against other bromodomains had been improved or not (compare matched molecular pair **23** and **24**). As the angle of the methyl groups is not the same across GNE-371 and compound **18** bound to TAF1(2), the incorporation of the 1-butenyl chain presumably forces the naphthyridone template to shift in the TAF1(2) binding site which results in a less complimentary fit and concomitant drop in affinity.

Despite this disappointing result with the 1-butenyl group, further characterization of compounds **20** and **23** was undertaken to determine the broader bromodomain selectivity profile. Single shot screening at 10 μM in the DiscoverX BROMO*scan* assay (Figure 4) was followed up by 11-point dose response curves of selected bromodomains where substantial activity was observed (Table 3). Importantly considering the compound genesis, activity against ATAD2 remained low (**20** %I = 47% and **23** %I = 24% at 10 μM, see supporting information Table S3 for more details).

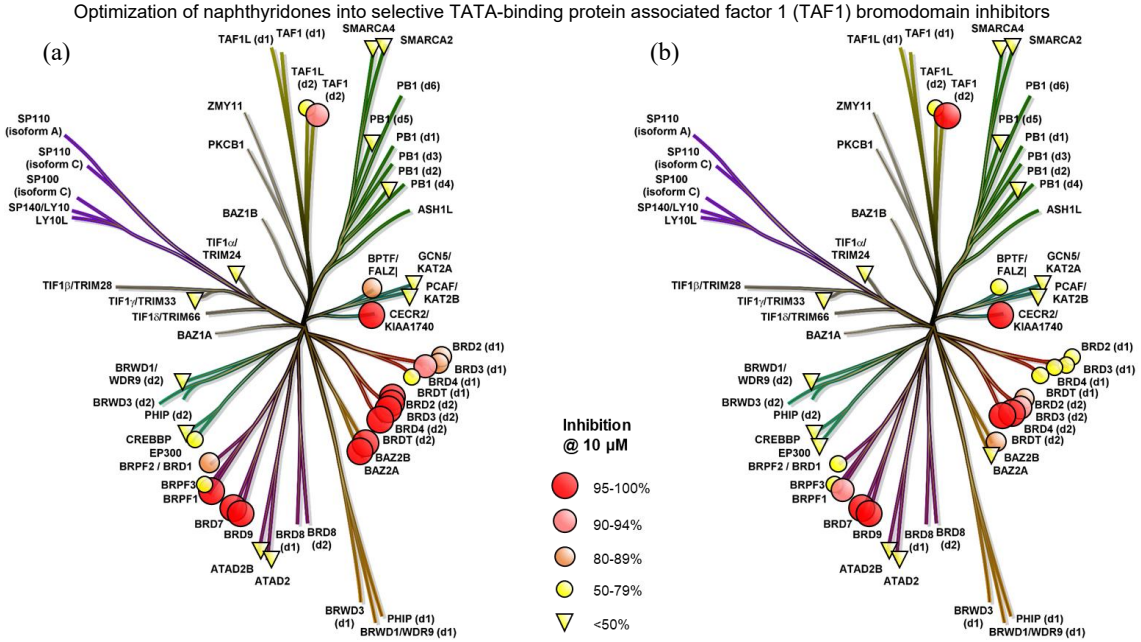


Figure 4. BROMOscan profiling at 10 μM for a) compound **20**; b) compound **23**.

Table 3. Full Curve BROMOscan Profiling of Compounds 20 and 23[a]

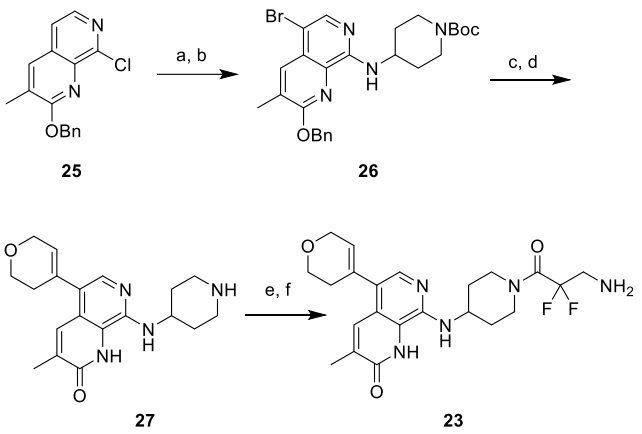
Compound	TAF1(2) pK_i	BRD4(1) pK_i	BRD4(2) pK_i	BAZ2B pK_i	BRD9 pK_i	CECR2 pK_i	BRPF1 pK_i	BRD1 pK_i
20	7.3	<5.3 (>×100)	5.8 (×30)	n.d.	6.3 (×100)	n.d.	6.0 (×100)	<5.3 (>×100)
23	9.1	<5.3 (>×6310)	6.1 (×1000)	6.0 (×1260)	7.4 (×50)	6.0 (×1260)	5.3 (×6310)	n.d.

[a]TAF1(2) fold selectivity is shown in brackets after the pKi value

Both compounds showed a reasonable single shot selectivity profile across the broader bromodomains with the more TAF1(2) potent **23** with the more encouraging profile. This was borne out when moving to the full curve assays with compound **23** showing ≥1000 fold selectivity over BRD4 and >50 fold selectivity over the broader bromodomains tested. Although not measured, it is expected that both **20** and **23** are active against TAF1L(2). With **23** meeting the predefined bromodomain selectivity criteria together with encouraging lipophilicity, solubility and permeability, attention turned to the demonstration of TAF1(2) cellular target engagement. A nanoBRET assay was generated in HEK-293 cells measuring the displacement of NanoLuc-tagged TAF1(2) from Halo-tagged histone H4 (Promega).[17, 34] However, **23** appeared inactive at the concentrations tested with a TAF1(2) nanoBRET pIC_{50} <5. In contrast, **20** displayed a TAF1(2) nanoBRET pIC_{50} 5.5, despite being 63-fold less potent against TAF1(2) in a biochemical binding assay compared with **23** (Table 3). The improved passive permeability of **20** appears to be critical to driving measurable activity in the nanoBRET assay (compound **20** AMP: 220 nm/s, compound **23** AMP: 67 nm/s). Active compound efflux may also be a factor but attempts to unravel this have thus far been unsuccessful.

The detailed synthetic routes to compounds **7**-**24** are described in the Supporting Information and the preparation of compound **23** is shown in Scheme 1. Previously described **25**[24] was reacted under Buchwald amination conditions followed by chemoselective bromination with NBS to give flexible intermediate **26**. Suzuki-Miyaura coupling with the commercially available dihydropyran pinacol boronate was followed by global deprotection to give piperidine **27**. Amide coupling and then acid mediated Boc deprotection proceeded in a 10% yield over the two steps to provide **23**.

Scheme 1. Synthesis of compound 23[a]



[a]Reagents and conditions: (a) 1-Boc-4-aminopiperidine, $Pd_2(dba)_3$, BrettPhos, NaO^{t}Bu, THF, 60 ºC, 4 h, 72%; (b) NBS, $CHCl_3$, rt, 1.5 h, 99%; (c) DHP-Bpin, $Pd(OAc)_2$, CataCXium A, K_2CO_3, 1,4-dioxane/H_2O, microwave 100 ºC, 1 h, 86%; (d) TFA, reflux, 3 h, 95%. (e) HATU, DIPEA, DMF, rt, 15 min-5 h, 26%; (f) 4 M HCl in 1,4-dioxane, rt, 2 h, 40%.

In summary, the optimization of unselective naphthyridone **7** into potent and TAF1(2) selective **23** has been achieved via crystallography guided structure-based drug-design to target Asp1539 with a pendant amine. This salt bridge interaction with Asp1539 drove selectivity over BRD4(1) and ATAD2, with selectivity further enhanced by the introduction of a

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semi-saturated dihydropyran. Careful modulation of the pKa was critical to drive permeability with cellular target engagement demonstrated with less selective, yet more permeable **20**. Although none of the molecules described meet the criteria of a chemical probe, compounds **20** and **23** represent interesting leads for the future development of small and/or bifunctional molecules to interrogate the biological function of TAF1(2).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Additional text describing all biochemical methods and results, all chemistry experimental procedures, BROMOscan data for **20** and **23**, docking methods, and X-ray data collection and refinement statistics

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): All authors except M.A.C and N.C.O.T. are current or former employees of GlaxoSmithKline.

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ABBREVIATIONS

AMP, artificial membrane permeability; ATAD2, ATPase family, AAA domain containing 2A; ATAD2B, ATPase family, AAA domain containing 2B; BAF, BRG1/BRM-associated factor; BAZ2A, bromodomain adjacent to zinc finger domain 2A; BAZ2B, bromodomain adjacent to zinc finger domain 2B; BCP, bromodomain containing protein; BD, bromodomain; BET, bromodomain and extra terminal domain; BRD1, bromodomain containing protein 1; BRD2, bromodomain containing protein 2; BRD3, bromodomain containing protein 3; BRD4, bromodomain containing protein 4; BRD7, bromodomain containing protein 7; BRD8, bromodomain containing protein 8; BRD9, bromodomain containing protein 9; BRDT, bromodomain containing protein, testis-specific; BRPF1, bromodomain and PHD finger-containing protein 1; BRPF3, bromodomain and PHD finger-containing protein 3; CAD, charged aerosol detection; CECR2, cat eye syndrome chromosome region candidate 2; CLND, chemiluminescent nitrogen detection; CREBBP, CREB binding protein; EP300, E1A-associated protein p300; FALZ, bromodomain PHD finger transcription factor; GCN5L2, general control non-depressible 5; KAc, acetylated lysine; LCMS, liquid chromatography mass spectrometry; LE, ligand efficiency; PBAF, polybromo-associated BAF; PBRM1, polybromo 1; PCAF, P300/CREBBP associated factor; PEPPSI, pyridine-enhanced precatalyst preparation stabilization and initiation; PHD, plant homeodomain; pIC_{50}, $-\log10(IC_{50})$; SMARCA2, SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily A, member 2; SMARCA4, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4; TRIM24, tripartite motif containing 24; TRIM33, tripartite motif containing 33; WDR9, WD repeat-containing protein 9.

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